Catal Lett (2009) 127:239–247
DOI 10.1007/s10562-008-9667-2

Preparation, Characterization and Catalytic Activities of Immobilized Enzyme Mimics

István Szilágyi · Ottó Berkesi · Mónika Sipiczki · László Korecz · Antal Rockenbauer · István Pálinkó

Received: 18 July 2008 / Accepted: 11 September 2008 / Published online: 7 October 2008

Abstract In order to find highly active and selective oxygen-transfer catalysts with appreciable durability, Cu(II)–histidine complexes were covalently grafted onto a chlorinated polystyrene resin as copper-containing enzyme mimics. The Cu(II)-histidine complexes and the mobile polymer were to resemble the active center and the proteomic skeleton of the enzymes, respectively. The resulting heterogenized complexes were expected to be nearly so active and more durable catalysts that are easier to recycle than their homogeneous counterparts. The substances were tested in a superoxide radical anion dismutation reaction. Control for the syntheses was exerted by protecting either the N-terminal or the C-terminal of the covalently grafted L-histidine molecules. During the preparative work generally applied methods of synthetic organic chemistry (alkylation or esterification) were used. Various anchored complexes were prepared and characterized by classical analytical methods, different forms of spectroscopy as well as molecular modeling. The covalently grafted complexes having the protected amino acids as ligands displayed remarkably high activities in the superoxide dismutase (SOD) test reaction.

Keywords Covalent grafting · Cu(II)-histidine derivative complexes · Polystyrene support · SOD activity

I. Szilágyi
Department of Inorganic and Analytical Chemistry, University of Szeged, Szeged 6720, Hungary

O. Berkesi
Department of Physical Chemistry, University of Szeged, Rerrich B. tér 1, Szeged 6720, Hungary

M. Sipiczki · I. Pálinkó (✉)
Department of Organic Chemistry, University of Szeged, Dóm tér 8, Szeged 6720, Hungary
e-mail: palinko@chem.u-szeged.hu

L. Korecz · A. Rockenbauer
Chemical Research Center, Institute of Structural Chemistry, P.O. Box 17, Budapest 1525, Hungary

1 Introduction

Superoxide dismutase (SOD) enzymes have important role in living systems. They, in cooperation with the catalase enzymes, provide defense from the detrimental effect of the superoxide radical anion [1]. The SOD enzymes dismutase the radical anion to dioxygen and hydrogen peroxide [1, 2]. The latter product is further transformed by the catalase enzymes to dioxygen and water. On the whole these enzymes are active in oxygen transfer reactions.

Enzymes are perhaps the most active but definitely the most selective catalysts known today. Since the vast majority of chemical industry is based on catalytic reactions, learning about the activity and selectivity influencing factors in catalytic systems are of utmost importance. The accumulated knowledge may lead researchers to invent novel, more efficient catalysts. For this invention a promising way is trying to mimic the enzymes. Mimicking may involve structural and/or functional modeling the active sites, usually done by preparing complexes having the appropriate metal ion(s) and ligands. If structural modeling is accurate enough, the appropriate function usually emerges. However, the activity and/or the selectivity may lie far away from those parameters of the native enzyme. There may be several reasons for this. Let us mention just some, considered to be important: (i) the geometry around

the metal ion(s) in the model may differ from that in the enzyme, (ii) the possibility of even slight structural distortion during catalysis is limited, (iii) the proteomic skeleton, being flexible, thus, having crucial role in defining the best conformation for the immediate surrounding of the active site, is fully missing. These shortcomings may be avoided if suitable geometric arrangement can be approached and a support is found being able to approximate the major properties of the proteomic skeleton [3, 4].

Quite often, especially in enzymes facilitating oxygen transfer, the active sites contain copper ion surrounded by amino acids of the proteomic skeleton. This is the case for a type of the SOD enzyme found in eukaryotic cells [5] (Fig. 1).

The active site contains Cu(II) as well as Zn(II) ions, however, only the copper ion has catalytic role, the zinc ion is "only" responsible for keeping the integrity of the structure through holding the bridging histidine molecule, when it is temporarily departed from the copper ion during the catalytic cycle [6]. Since the amino acid ligands are L-histidine molecules copper–histidine complexes may be good structural and probably functional approximations of the active site of the Cu, Zn–SOD enzyme. Indeed, we have found that Cu(II)-histidine displayed SOD activity, however, it was two and a half magnitudes lower than that of the native enzyme [7, 8]. Even if the complex itself was a good homogeneous catalyst, recovery might be difficult if it was possible at all. Anchoring the complex by various methods (adsorption-hydrogen bonding [9], ion exchange [8–10], covalent grafting [9]) onto rigid supports (montmorillonite [8, 9], silica gel [9, 11] or zeolite [10]) is a solution to this problem—a solid catalyst is always easier to handle than a homogeneous one. When it was done by the ion-exchange method a more durable catalyst was obtained with the advantage of being easily recoverable, however, the SOD activity was worse than that of the support-free complex [8].

In search for immobilized Cu(II)-histidine derivative complexes with higher SOD activities the rigid support have been changed to a more flexible polymer and the ion-exchange method of immobilization to covalent grafting. Covalent grafting involved the anchoring of appropriately protected L-histidine molecules onto the polymer (controlled anchoring onto the swellable resin) and then, building the copper complexes either without deprotection or after it. The SOD activities of these materials were tested and the structures of the best catalysts were elucidated.

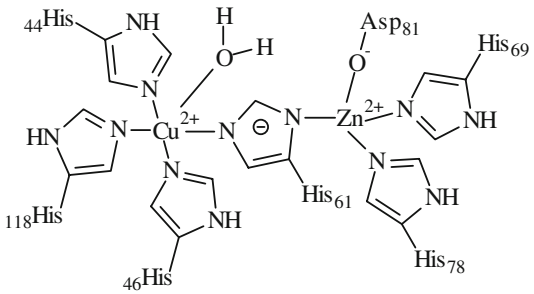


Fig. 1 The active site of the Cu, Zn–SOD enzyme

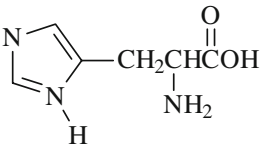
2 Experimental

2.1 Materials

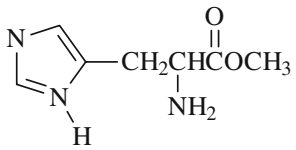
The central ion for the complexes was Cu^{2+} and the ligands (products of Aldrich Co.) were either L-histidine (H–His–OH), *tert*-butoxycarbonyl-L-histidine (BOC–His–OH) or L-histidine methyl ester (H–His–OMe) (Fig. 2). The source of Cu^{2+} ions was the aqueous solution of $Cu(NO_3)_2$—product of Reanal. The amino acids were used as received.

The host material was a chlorinated polystyrene resin [poly(styrene-co-vinylbenzyl chloride-co-divinyl-benzene with 3.8–4.2 mmol/g chlorine content] from Aldrich, abbreviated as PS–PheCH_2Cl in the followings (Fig. 3).

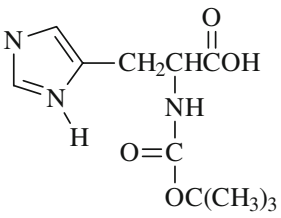
Further materials used: cc. (96%) sulfuric acid (RPE; Farmitalia Carlo Erba S.p.A.), methylene chloride, trifluoroacetic acid (Aldrich Co.), solid NaOH, isopropanol, toluene (all from MOLAR CHEMICALS Ltd.).



(L)-H-His-OH

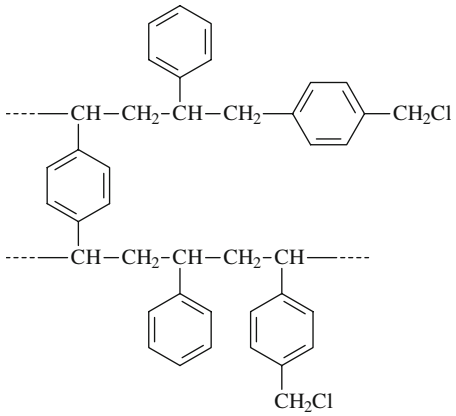


(L)-H-His-OMe



(L)-BOC-His-OH

Fig. 2 The molecules to be immobilized



PS-PheCH$_2$Cl

Fig. 3 The chlorinated polystyrene resin host

Chemicals used throughout the work were high (over 99.5%) purity materials and were used without further purification.

2.2 Preparation of the Covalently Grafted Complexes

The first step of immobilization was the reaction of the appropriately protected amino acid with the chlorinated polystyrene. The general method is as follows: certain amount of functionalized resin was suspended in isopropanol and calculated amount of protected amino acid solution was added. Coupling with the ester or the BOC-amino acid was achieved by refluxing the mixture under basic conditions during constant stirring. After 3 h the solid substance was filtered washed several times and dried. The resulting material was divided into two parts. The first one was left unchanged, the other one was either treated with sulfuric acid, in order to hydrolyze the ester bond or was refluxed under vigorous stirring at moderate temperature (338 K) for 2 h in a 1:1 mixture of CH_2Cl_2 and CF_3COOH in order to remove the BOC protecting group. Then, the samples (four different substances) were soaked in $Cu(NO_3)_2$ solution under stirring overnight. After filtering, solution of the appropriate amino acid derivatives (i.e., either with or without protecting group) was added in excess. The suspension was refluxed for an hour and stirred for 4–5 more hours at room temperature. Finally, the solid material was filtered, rinsed with isopropanol 5–6 times, dried and stored in a vacuum desiccator.

Since the resin was reported to be amine selective, anchoring of histidine and then complexation were performed with the unprotected amino acid as well.

2.3 The Reaction for Probing the SOD Activity

The SOD activity was investigated by the method of Beauchamp and Fridovich [12]. Its description is as follows. On illumination under aerobic conditions riboflavin is reduced by L-methionine, and the reduced form reacts with oxygen forming a peroxide derivative, which after decomposition provides with the superoxide radical anion. The ions are captured by the nitro blue tetrazolium (NBT). This compound changes color upon the reaction (reduction occurs): the original yellow color turns blue.

The transformation can be followed by spectrophotometry, measuring the absorbance at 560 nm. When enzyme or enzyme mimic is present, it captures the superoxide radical ion, consequently, the photoreduction of NBT is inhibited, i.e. the enzyme or its mimic works the better when the color change (measured by the absorbance) is the smaller. The SOD probe reaction was carried out at room temperature in an aqueous solution (host-free complex) or suspension (immobilized complex) at pH = 7 ensured with a phosphate buffer. The reaction mixture contained 0.1 cm^3 of 0.2 mmol/dm^3 riboflavin, 0.1 cm^3 of 5 mmol/dm^3 NBT, 2.8 cm^3 of 50 mmol/dm^3 phosphate buffer (Na_2HPO_4 and KH_2PO_4) containing EDTA (0.1 mmol/dm^3) and L-methionine (13 mmol/dm^3) and the catalyst. Riboflavin was added last and the reaction was initiated by placing the tubes under two 15 W fluorescent lamps. It was allowed to run for 10 min to reach equilibrium. The role of EDTA is to remove the disturbing trace metal ions, since the metal ion–EDTA complexes have no SOD activity. From the resulting graph the volume of enzyme mimicking complex corresponding to 50% inhibition was registered to allow a comparison with the efficiency of the real enzymes and other SOD mimics. There was no reaction without illumination and the pure resin did not display SOD activity either.

2.4 Methods of Structural Characterization

The amount of copper(II) on/in the solid host was measured by atomic absorption spectrometry (AAS—Perkin Elmer 3110 instrument). Before measurements the solid materials were dissolved in *aqua regia*.

The nitrogen content was determined by the Kjeldhal method. The nitrogen content of the samples was turned to NH_3 by the sequential addition of cc. H_2SO_4 and NaOH solution. The ammonia formed was reacted with boric acid, then, it was titrated with aqueous HCl of known concentration.

Photoacoustic spectra (PAS) were recorded on a BIO-RAD Digilab Division FTS-65A/896 FT-IR spectrometer equipped with an MTEC 200 photoacoustic detector. The 4000 cm^{-1}–400 cm^{-1} range was investigated. The resolution was 4 cm^{-1}. For a spectrum 256 interferograms were collected. Samples were loaded into a sample holder with 3 mm diameter. Measurements were performed under He atmosphere.

One advantage of using photoacoustic detector for recording the spectra is that the solid material has not to be pressed into a tablet or pellet, thus, heat evolving during pressurization does not destroy our modified resin substances. The other one is the information from the interior of the sample unavailable to other IR methods.

Raman spectra were taken on a dedicated Bio-Rad Digilab Division FT-Raman spectrometer using the 1064 nm line of a T-10-106c type Nd:YVO_4 laser from Spectra Physics. The spectra of the solid samples were recorded in a 5 mm NMR tube with 4 cm^{-1} optical resolution and accumulating 128 scans. Spectra were recorded in the 3600 cm^{-1}–100 cm^{-1} region.

Both spectrum types were evaluated with the Win-IR package.

The EPR spectra were recorded at 298 K on a Brucker Elexys 500 X-band spectrometer equipped with NMR Gauss-meter and frequency counter with 100 kHz field modulation. The EPR parameters were calculated with a computer program [13].

3 Results and Discussion

3.1 Preparation of the Covalently Anchored Cu(II)-amino acid complexes

Since the chlorine functionality is attached to a benzylic group in our host polymer, coupling reactions between the resins and the amino acids are expected to be relatively easy. To exert control on the syntheses protected amino acids were coupled first. Coupling of H–His–OMe resembles *N*-alkylation (PS–PheCH_2–His–OMe), while that of BOC–His–OH is an esterification (BOC–His–OCH_2Phe–PS).

Then, the respective materials were divided into two parts and the amino acids were deprotected from one half. Thus, four samples were obtained, two having the covalently grafted N– or C–protected histidine (BOC–His–OCH_2Phe–PS and PS–PheCH_2–His–OMe, respectively) two having the deprotected amino acids ($^{[BOC]}$H–His–OCH_2Phe–PS and PS–PheCH_2–His–$OH^{[OMe]}$).

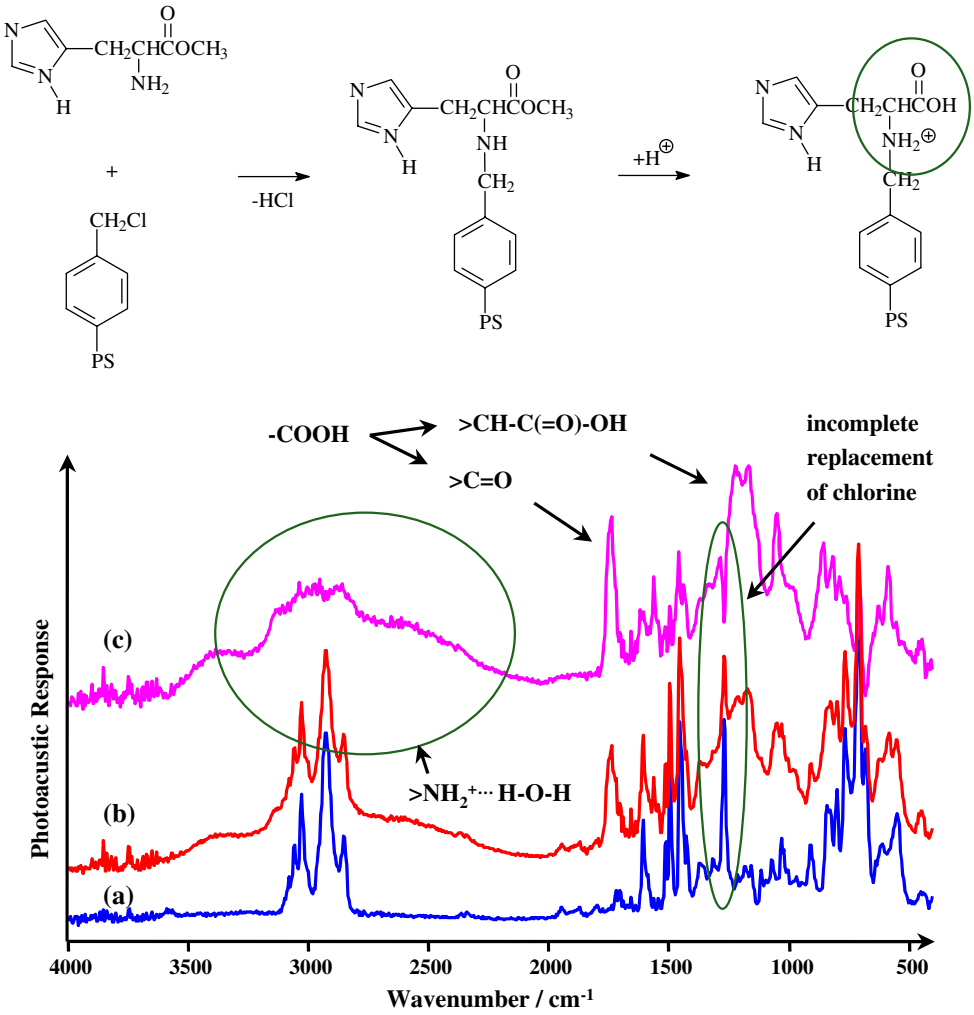


Fig. 4 The anchoring and deprotection procedures of the C-protected histidine molecule and the PAS FT-IR spectra of (**a**) the polystyrene host, (**b**) the covalently grafted and deprotected amino acid and (**c**) their difference spectrum

After having soaked the samples in copper(II) solution excess of the corresponding amino acids were added.

The same procedure was applied using (unprotected) L-histidine.

Let us remark that choosing isopropanol as solvent was important. The syntheses were not successful in water: the isolated materials were white or yellowish. Upon applying isopropanol greenish or bluish materials could be obtained.

In order to make sure that we had control over the covalent grafting procedure it was followed by vibrational spectroscopy. The PAS FT-IR spectra together with reaction schemes of anchoring and deprotecting (where applicable) the amino acids are depicted in Figs. 4, 5, 6. The vibrations corresponding to the encircled typical parts in the schemes are also encircled in the spectra.

It is clear that the intended reactions proceeded, i.e., there was control over the grafting procedure, indeed. The amino acids could be appropriately anchored, even if chlorine replacements were incomplete. Moreover, Fig. 5 reveals that the resin was amine selective indeed: covalent grafting of the unprotected amino took place through the amino group.

If we go further and compare the PAS FT-IR (Fig. 7) and the FT-Raman (Fig. 8) spectra of the covalently grafted copper complexes starting from the three different amino acids (C–, N–protected after deprotection or unprotected), it is to be seen that whatever end (C– or N–terminal) of the amino acids were anchored initially, the structures of the resulting complexes were very similar.

Although we cannot be definite about the accurate number and types of coordinating groups in the complexes, the spectra as well as the methods of syntheses limits the possible structures. The ring nitrogens of the imidazole units are the most probable coordination sites from the polymer side having the covalently grafted amino acids (denoted as modified polymer in the followings). The imidazole nitrogen and/or the carboxylate oxygen might coordinate to the copper ion when excess amino acid was given to the synthesis mixture. Spectra in Fig. 8 reveal that the amino groups were protonated, therefore, they probably did not coordinate.

These were the findings with the deprotected and the unprotected amino acid ligands. By analogy the imidazol nitrogens should be the coordination sites from the polymer side having the covalently grafted protected amino acid. The C-protected amino acid excess might coordinate through the imidazole nitrogen and/or the amino nitrogen, while the N-protected excess may do it through its imidazole nitrogen and/or the carboxylate group.

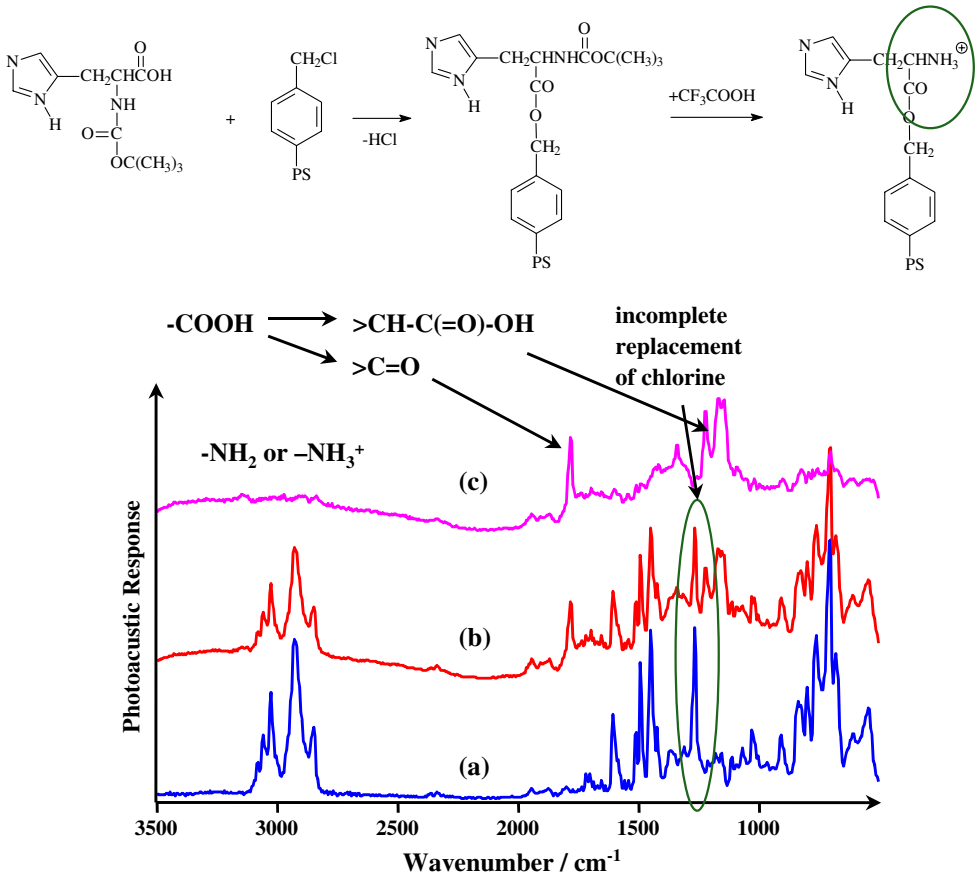


Fig. 5 The anchoring and deprotection procedures of the N-protected histidine molecule and the PAS FT-IR spectra of (**a**) the polystyrene host, (**b**) the covalently grafted and deprotected amino acid and (**c**) their difference spectrum

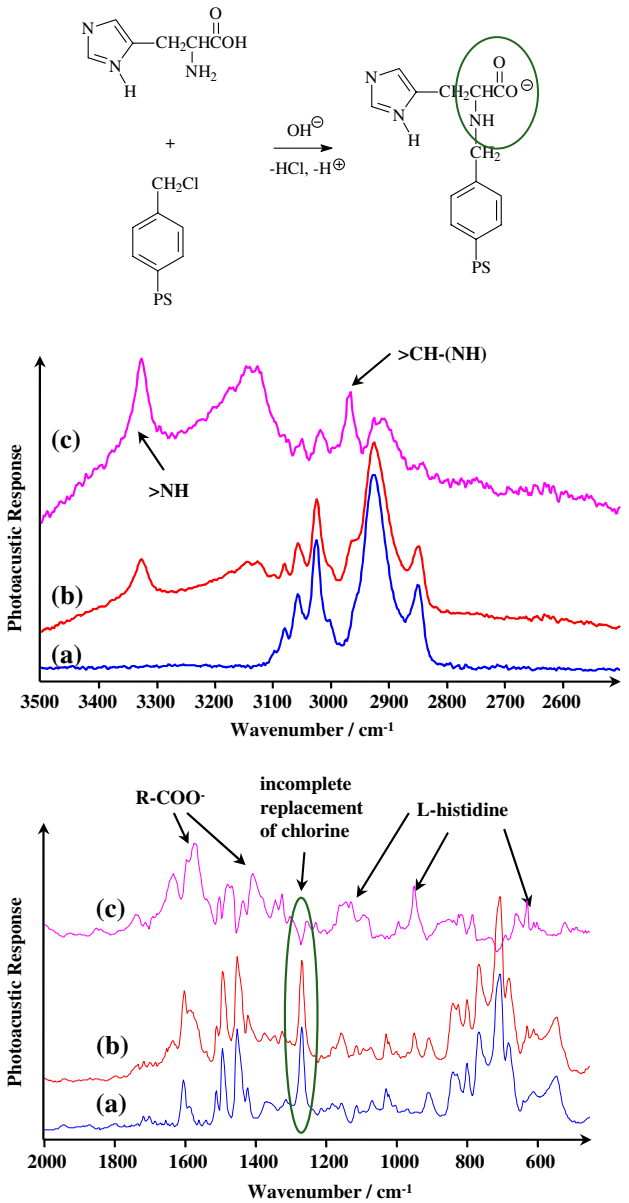


Fig. 6 The anchoring procedure of the (unprotected) histidine molecule and the PAS FT-IR spectra of (**a**) the polystyrene host, (**b**) the covalently grafted amino acid and (**c**) their difference spectra

3.2 The SOD Activities of the Samples

Since testing of SOD activities is fast, it was done with all samples before entering further structural characterization. It turned out that those samples having the protected amino acids as ligands (BOC–His–OH–Cu(II)–BOC–His–OCH_2-Phe–PS and PS–$PheCH_2$–His–OMe–Cu(II)–H–His–OMe) displayed outstanding and very similar SOD activities (Table 1, rows 5 and 6). Although the other three samples were also active (PS–$PheCH_2$–H–His–OH–Cu(II)–H–His–OH, H–His–OH–Cu(II)–$^{[BOC]}$H–His–OCH_2Phe–PS and PS–$PheCH_2$–H–His–$OH^{[OMe]}$–Cu(II)–H–His–OH) the IC_{50} values were much higher, i.e. the SOD activities were much lower (Table 1, rows 7–9). Two of them were even less active than the support-free complex (Cu(II)–H–His–OH, Table 1, row 2). Nevertheless, covalent grafting supplied better performing immobilized enzyme mimics than anchoring *via* electrostatic forces onto a rigid support (Cu(II)–H–His–OH—montmorillonite, Table 1, row 3). However (and not very surprisingly), the native enzyme was still the most active (Cu, Zn–SOD, Table 1 row 4).

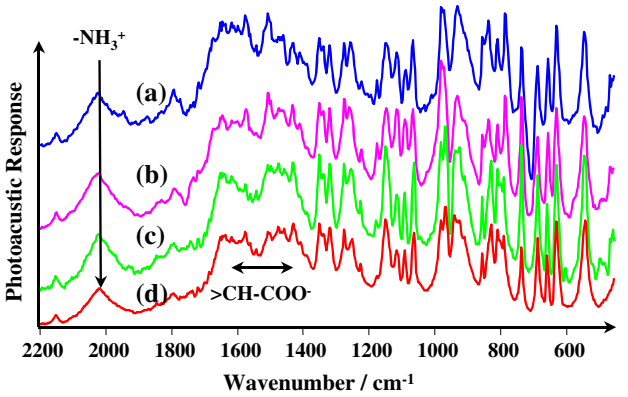


Fig. 7 The stacked PAS FT-IR spectra of the anchored (**a**) N-protected and (**b**) C-protected histidine molecules after deprotection, (**c**) the anchored unprotected histidine and (**d**) the support-free histidine molecules

Possibly, the samples with high activities approximate better the geometry of the active site in the native enzyme than the others and the immediate environment (the geometry as well as the coordinating moieties) should be very similar in these materials.

3.3 Structural Characterization of Immobilized Materials

During further structural characterization we were concentrating on the two most active samples, BOC–His–OH–Cu(II)–BOC–His–OCH_2Phe–PS and PS–$PheCH_2$–His–OMe–Cu(II)–H–His–OMe substances they are.

Atomic absorption spectroscopy and the measurement of nitrogen content allowed the determination of the concentrations of copper ions and the protected amino acids immobilized on the polymer, respectively. They were 0.026678 mmol/g Cu(II) and 0.1054 mmol/g BOC-protected histidine for BOC–His–OH–Cu(II)–BOC–His–OCH_2Phe–PS and 0.03176 mmol/g Cu(II) and 0.1258 mmol/g methyl ester-protected histidine for PS–$PheCH_2$–His–OMe–Cu(II)–H–His–OMe. The amino acid/Cu(II) ratios are close to 4 giving this as the number of coordinating amino acid molecules in both immobilized complexes. This arrangement differs from that either in the support-free complex [15–17], the Cu(II)–histidine complex anchored to montmorillonite with electrostatic forces

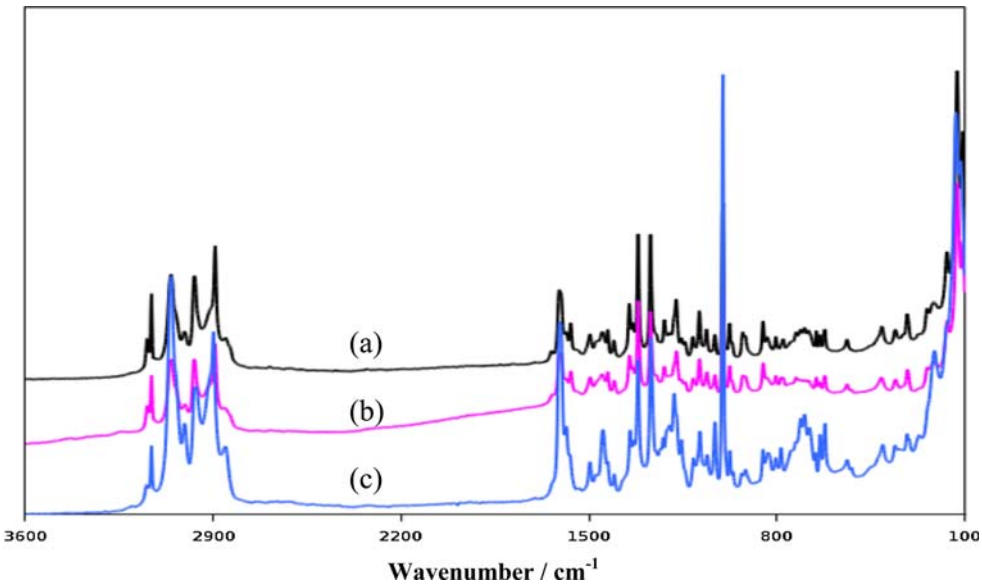


Fig. 8 The stacked FT-Raman spectra of the anchored (**a**) N-protected and (**b**) C-protected histidine molecules after deprotection and (**c**) the anchored unprotected histidine molecules

Table 1 The IC_{50} values of the immobilized complexes, the support-free complex and the native enzyme

Materials	IC_{50} (μM)	Reference
Cu(II)–H–His–OH	108.6	[8]
Cu(II)–H–His–OH–montmorillonite	251.4	[8]
Cu, Zn–SOD	0.4	[14]
BOC–His–OH–Cu(II)–BOC–His–OCH_2Phe–PS	13.4	This work
PS–PheCH_2–His–OMe–Cu(II)–H–His–OMe	10.4	This work
PS–PheCH_2–H–His–OH–Cu(II)–H–His–OH	199.4	This work
H–His–OH–Cu(II)–$^{[BOC]}$H–His–OCH_2Phe–PS	61.5	This work
PS–PheCH_2–H–His–$OH^{[OMe]}$–Cu(II)–H–His–OH	127.7	This work

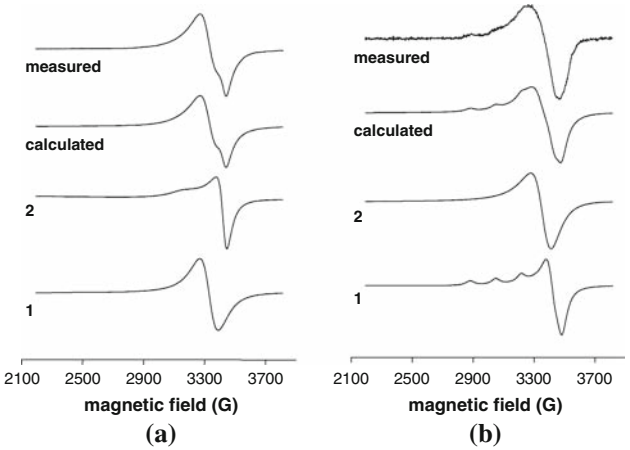


Fig. 9 The measured (298 K), the simulated and the decomposed EPR spectra of (**a**) PS–PheCH_2–His–OMe–Cu(II)–H–His–OMe and (**b**) H–His–OH–Cu(II)–$^{[BOC]}$H–His–OCH_2Phe–PS

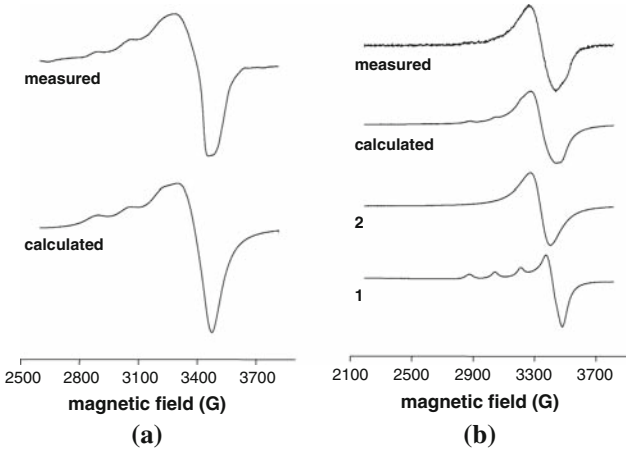


Fig. 10 The measured (298 K), the simulated and the decomposed EPR spectra of (**a**) BOC–His–OH–Cu(II)–BOC–His–OCH_2Phe–PS and (**b**) PS–PheCH_2–H–His–$OH^{[OMe]}$–Cu(II)–H–His–OH

[8] or encapsulated in saponite [18] or NaY [19–21]. In the complexes referred chelating occurred: two amino acids were coordinated to the central copper(II) ion. However, the coordination of four amino acids resembles that of the environment of the copper(II) cofactor in the SOD enzyme, no wonder that both polymer-supported complexes displayed higher SOD activities than the montmorillonite intercalated one or the unsupported complex.

Table 2 EPR spectral parameters

Materials	Comp. (%)	g_o	$G_\perp$	$g_\parallel$	A_o	$A_\perp$	$A_\parallel$
Cu, Zn–SOD[a]	100	2.13	2.07	2.26	–	–	–
Cu(II)–H–His–OH[b]	100	2.12	2.11	2.15*	–	–	–
PS–PheCH$_2$–H–His–OH$^{[OMe]}$–Cu(II)–H–His–OH	(1) 38.2	2.09	2.06	2.25	56.7	3.1	164.0
	(2) 61.8	2.09	–	–	–	–	–
PS–PheCH$_2$–His–OMe–Cu(II)–H–His–OMe	(1) 27.8	2.12	2.06	2.24	–	–	–
	(2) 72.2	2.15	–	–	–	–	–
H–His–OH–Cu(II)–$^{[BOC]}$H–His–OCH$_2$Phe–PS	(1) 19.9	2.12	2.06	2.25	59.5	7.2	164.0
	(2) 80.1	2.10	–	–	–	–	–
BOC–His–OH–Cu(II)–BOC–His–OCH$_2$Phe–PS	100	2.14	2.08	2.25	71.8	26.3	162.7

The Cu^{63} couplings are given in G units

[a] [21]

[b] [8]

* Estimated from the shape of the signal (the value is somewhat vague)

EPR spectra may be of help in further elucidating structural details. Beside the spectra of the two most active substances those of the immobilized materials having the deprotected amino acid ligands were also measured. Although the spectra were taken at room temperature they show anisotropic character indicating that ligand mobility was low. In all except one case the measured spectra could be decomposed into two components (Figs. 9 and 10).

The EPR spectrum of the BOC–His–OH–Cu(II)–BOC–His–OCH$_2$Phe–PS sample indicates the presence of only one species (Fig. 10a). Let us recall that this and the PS–PheCH$_2$–His–OMe–Cu(II)–H–His–OMe sample displayed outstanding SOD activities.

Spectral parameters of the component spectra (where relevant) and the relative quantities of the corresponding species together with the parameters of the unsupported Cu(II)–H–His–OH complex [8] and the native Cu, Zn–SOD enzyme [22] are summarized in Table 2.

Comparing the parameters of the enzyme to those of the component spectra of the anchored complexes as well as visual inspection showed that the **#1** component spectrum of PS–PheCH$_2$–His–OMe–Cu(II)–H–His–OMe and the spectrum of BOC–His–OH–Cu(II)–BOC–His–OCH$_2$Phe–PS resembled each other and those of the enzyme the best indicating similar structural arrangements. It also has to be noted that the parameters are very different from the parameters and the spectra of the support-free complex. The high g_0 value is a sign of having three nitrogens in one plane. In our view the fourth coordinating atom lying above the plane is also a nitrogen atom. The first two from the modified polymer side are imidazole nitrogens, and so do the other two coordinating moieties. The amino nitrogens and the carboxylate oxygens are excluded since in spite of the different ways of protection the **#1** component spectrum of PS–PheCH$_2$–His–OMe–Cu(II)–H–His–OMe and the spectrum of BOC–His–OH–Cu(II)–BOC–His–OCH$_2$Phe–PS are very similar. Thus, the structural arrangement in these two immobilized materials thought to be responsible for the outstanding SOD activity is tetrahedral distorted to different extents with four imidazol nitrogens as coordinating moieties. The extent of distortion is probably due to the different steric requirements of the protecting groups. They may also influence the accessibility of the central ion during the dismutation test reaction.

The other (**#2**) component spectrum of PS–PheCH$_2$–His–OMe–Cu(II)–H–His–OMe and the component spectra of the anchored complexes with deprotected ligands, have different steric arrangements. Lower g_0 values indicate four nitrogen atoms in the equatorial plane or the carboxylate oxygen(s) in the third and/or fourth coordination positions. Whatever the accurate steric structures are those immobilized complexes displayed much lower SOD activities.

4 Conclusions

Immobilized Cu(II)–L-histidine complexes were prepared by covalently grafting the amino acid ligands onto chlorine-functionalized polystyrene. Control on the syntheses was exerted by applying protected amino acids, although, the resin displayed amine selectivity. Two anchored complexes, those having the protected amino acids as ligands displayed outstanding SOD activities. In these substances, irrespective to the anchoring groups these complexes assumed to more or less distorted tetrahedral geometries and one ring nitrogen of all four histidine molecules was coordinated to the central ion.

Acknowledgment This work was supported by the National Science Fund of Hungary through grant K62288. The financial help is highly appreciated.

References

1. Malström BG, Andreasson LE, Reinhammer B (1975) In: Enzymes Academic Press, New York, p 533
2. Tainer JA, Getzoff ED, Richardson JS, Richardson DC (1983) Nature 306:284
3. Piacham T, Ayudhya CIN, Prachayasitticul V, Bulow L, Ye L (2003) Chem Commun 1254
4. Bellot F, Hardre L, Pelosi G, Therisod M, Policar C (2005) Chem Commun 5414
5. Holm RH, Kennepohl P, Solomon E (1996) Chem Rev 96:2239
6. Dupeyrat F, Vidaud C, Lorphelin A, Berthomieu C (2004) J Biol Chem 279:48091
7. Weinstein J, Bielski BHJ (1980) J Am Chem Soc 102:4916
8. Szilágyi I, Labádi I, Hernadi K, Kiss T, Pálinkó I (2005) Stud Surf Sci Catal 158:1011
9. Jakab IN, Hernadi K, Méhn D, Kollár T, Pálinkó I (2003) J Mol Struct 651–653:109
10. Mesu JG, Visser T, Beale AM, Soulimani F, Weckhuysen BM (2006) Chem Eur J 12:7167
11. Jakab IN, Hernadi K, Kiss JT, Pálinkó I (2005) J Mol Struct 744–747:487
12. Beauchamp C, Fridovich I (1971) Anal Biochem 44:276
13. Rockenbauer A, Korecz L (1996) Appl Magn Reson 10:29
14. Szilágyi I, Labádi I, Hernadi K, Pálinkó I, Fekete I, Korecz L, Rockenbauer A, Kiss T (2005) New J Chem 29:740
15. Sundberg RJ, Martin RB (1974) Chem Rev 74:471
16. Deschamps P, Kulkarni PP, Sarkar B (2004) Inorg Chem 43:3338
17. Deschamps P, Kulkarni PP, Gautam-Basak M, Sarkar B (2005) Coord Chem Rev 249:895
18. Fu L, Weckhuysen BM, Verberckmoes AA, Schoonheydt RA (1996) Clay Miner 31:491
19. Grommen R, Manikandan P, Gao Y, Shane T, Shane JJ, Schoonheydt RA, Weckhuysen BM, Goldfarb D (2000) J Am Chem Soc 122:11488
20. Weckhuysen BM, Verberckmoes AA, Fu L, Schoonheydt RA (1996) J Phys Chem 100:9456
21. Weckhuysen BM, Verberckmoes AA, Vannijvel IP, Pelgrims JA, Buskens PL, Jacobs PA, Schoonheydt RA (1995) Angew Chem Int Ed Eng 34:2652
22. Li D, Li S, Yang D, Yu J, Huang J, Li Y, Tang W (2003) Inorg Chem 42:6071